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IV. 研究成果の刊行物・別刷

Early Pulmonary Resection for *Mycobacterium Avium* Complex Lung Disease Treated With Macrolides and Quinolones

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Background. The purpose of this study was to examine the postoperative outcomes of patients with *Mycobacterium avium* complex (MAC) lung lesions persisting despite treatment with multiple antibiotics.

Methods. Patients with localized pulmonary lesions persisting despite extensive state-of-the art antimicrobial chemotherapy became candidates for surgical resection. Twenty-two patients who were expected to retain sufficient postoperative pulmonary function were included in this study. These patients received chemotherapy for 2 to 37 months (mean, 17). Surgical procedures were lobectomy (n = 15), segmentectomy (n = 4), and partial lung resection (n = 6). Three patients underwent bilateral resections.

Results. *Mycobacterium avium* complex causing bronchiectasis or cavitary lesions was detected preoperatively in all 22 patients. There was no major operative morbidity or mortality. Postoperative chemotherapy was continued for 6 to 35 months. All patients were alive and well at follow-ups ranging from 6 to 164 months (median, 46). Both vital capacity and forced expiratory volume in 1 second after surgery were maintained at 89% and 84% of the preoperative values, respectively. *Mycobacterium avium* complex disappeared from sputum after surgery in all patients. In 1 patient, 4 months after resection of a cavitary lesion, MAC-positive sputum presumed to be from the contralateral lung lesion became negative during continuation of chemotherapy.

Conclusions. The long-term outcomes of patients operated on for MAC resistant to antimicrobial chemotherapy were excellent. For such patients, we recommend surgery before the disease becomes exceedingly advanced and nonresectable. Additionally, in extensive disease, the excision of large cavitary bacterial foci may assist the medical management of contralateral lesions.

(Ann Thorac Surg 2006;81:2026–30)

The detection of nontuberculous mycobacterial lung diseases has improved as a result of advances in diagnostic methods [1, 2]. *Mycobacterium avium* and *Mycobacterium intracellurare*, generally referred to as the *Mycobacterium avium* complex (MAC), are the most common in Japan. Although MAC has been treated with multiple drugs, including clarithromycin or levofloxacin, cure with medications alone remains difficult to achieve, especially in patients with cavitary or bronchiectatic lesions. Therefore, surgical resection continues to play an important role in the management of this disorder. The aim of this study was to retrospectively examine the outcomes of patients who underwent pulmonary resections for MAC disease.

Patients and Methods

This study was carried out in accordance with the guidelines set by the Japanese Ministry of Health, Labor, and Welfare. The Institutional Review Board approved this study, and informed consent was waived. Surgical and medical records of all patients who underwent pulmonary resection for MAC pulmonary disease between January 1, 1990, and July 31, 2005, at Keio University Hospital were reviewed. Smears, cultures, and polymerase chain reaction examinations of sputum or bronchial washings were performed before surgery. Samples submitted to the microbiology laboratory were concentrated and decontaminated by standard methods. Smears were screened by both the fluorochrome method and Ziel-Nielsen staining. At the same time, samples were tested by an Amplicor Mycobacterium DNA detection kit (Roche Diagnostics, Tokyo, Japan), and cultured in a liquid medium (BBL MGIT; Becton Dickinson, Franklin Lakes, New Jersey). Microdilution antimycobacterial susceptibility test, BrothMIC, (Kyokuto

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Table 1. Clinical Characteristics of Patients

Patient	Age (years)/ Sex	Diagnostic Manifestation	Pretreatment (Duration, Months)	Main Lesion	Surgical Procedure	Posttreatment (Duration, Months)	Outcomes (Months)
1	34/M	Hemoptysis	INH+RFP+EB 20	Ectasis	R Low Lobectomy	INH+RFP+EB 6	164A
2	51/W	CXR	INH+RFP+CAM+OFLX 30	Ectasis[a]	R Mid Lobectomy+Up Wedge / L lingula segmentectomy+Up Wedge	INH+RFP+CAM 6	101A
3	59/W	Hemoptysis	CAM 12	Ectasis[a]	R Mid lobectomy+Up Wedge	RFP+CAM 12	78A
4	34/W	CXR	INH+RFP+CAM+LVFX 6	Cavity	R Up Wedge/L Up Wedge	INH+RFP+CAM+ LVFX 7	73A
5	61/W	Cough, sputum	RFP+INH+CAM 20	Ectasis[a]	R Mid Lobectomy+S6 Wedge	INH+RFP+CAM 7	69A
6	49/W	CXR, hemoptysis	INH+RFP+EB 24	Cavity	R S2 segmentectomy	CAM+EB 7	61A
7	57/M	Sputum	RFP+EB+CAM 6	Ectasis	R Low Lobectomy	RFP+EB 3	59A
8	61/W	CXR	LVFX+CAM 15	Ectasis[a]	R Mid Lobectomy	CAM+LVFX 6	59A
9	55/W	Sputum	INH+RFP+CAM 7	Ectasis	RS6 segmentectomy+Up Wedge	LVFX 6	59A
10	52/W	CXR	CAM+LVFX 7	Ectasis[a]	L Up Wedge/R Mid Lobectomy+Up Wedge	CAM+LVFX 5	51A
11	59/W	Sputum	RFP+EB+CAM+SPFX 37	Cavity	R Up Lobectomy+Low Wedge	RFP+EB+CAM+ SPFX_35	46A
12	41/M	CXR	INH+RFP+EB 24	Cavity	R Up Lobectomy	INH+RFP+EB_6	46A
13	63/M	CXR	EB+RFP+CAM 12	Cavity	R Up Wedge	EB+RFP+CAM 6	46A
14	77/M	Cough, sputum	INH+RFP+EB 2	Ectasis	R Up Wedge	INH+RFP+EB 6	36A
15	72/W	CXR	CAM 8	Ectasis[a]	R Mid Wedge	CAM 6	23A
16	58/M	CXR	EB+RFP+CAM 20	Cavity	R S1,2 segmentectomy	CAM 6	22A
17	53/W	Hemoptysis	CAM 33	Ectasis[a]	R Mid Lobectomy	CAM 9	20A
18	37/M	CXR	EB+RFP+CAM+LVFX 14	Cavity	R Up Lobectomy	EB+RFP+CAM+ LVFX 6	19A
19	54/W	Hemoptysis	INH+RFP+EB+SM+ PZA24_CAM32	Cavity	R Mid Lobectomy+Low Wedge	CAM 6	16A
20	60/W	Sputum	EB+RFP+CAM+LVFX+ KM24	Ectasis[a]	R Up+Mid Lobectomy	EB+RFP+CAM6	15A
21	71/W	CXR	CAM+LVFX 20	Ectasis[a]	R Mid Lobectomy+S6 segmentectomy	CAM 6	14A
22	30/W	CXR	EB+RFP+CAM+LVFX+ SM 14	Ectasis	R Up Lobectomy	CAM 6	6A

[a] Middle lobe or lingular type.

A = alive; CAM = clarithromycin; CXR = chest roentogenogram; EB = ethambutol; INH = isoniazid; KM = kanamycin; L = left; Low = lower lobe; LVFX = levofloxacin; M = man; Mid = middle lobe; OFLX = ofloxacilin; PZA = pirazinamid; R = right; RFP = rifampicin; SM = streptomycin; SPFX = suparfloxacin; Up = upper lobe; W = woman; Wedge = wedge resection.

Pharmaceutical Industrial, Tokyo, Japan) was performed to examine drug sensitivity in 9 recent cases. Minimum inhibitory concentrations of 10 drugs were measured.

The surgical indications in this series of patients were (1) MAC disease refractory to multiple drug therapy, including clarithromycin, rifampicin, ethambutol, or levofloxacin; (2) localized pulmonary lesions; and (3) sufficient predictive postoperative pulmonary function.

Results

Patient Population

Between January 1, 1990, and July 31, 2005, pulmonary resections for MAC were performed in 15 women and 7 men ranging in age between 30 and 77 years (mean, 54). The main characteristics of the patient population are shown in Table 1. No patients suffered from immunodeficient disorders, such as human immunodeficiency virus. One patient, however, had primary lung cancer combined with MAC pulmonary disease. All patients underwent lung resection to treat MAC disease and not for diagnostic purposes.

Preoperative Condition and Chemotherapy

Ten asymptomatic patients with abnormal findings on chest roentgenograms at health maintenance examinations were diagnosed with MAC disease. The other 12 patients had symptoms, including productive cough in 6

Table 2. Results of Pulmonary Function Test Before and After Surgery

	Total Cases (n = 18)			Lobectomy[a] (n = 11)		
	Before Surgery	After Surgery	*p* Value	Before Surgery	After Surgery	*p* Value
Vital capacity (L)	3.08 ± 0.77	2.74 ± 0.84	0.0001	2.98 ± 0.68	2.73 ± 0.82	0.007
Percent vital capacity (%)	99.4 ± 11.1	92.3 ± 14.2	0.02	95.6 ± 10.3	91.3 ± 14.4	0.21
FEV1.0 (L)	2.34 ± 0.67	1.97 ± 0.65	0.0001	2.33 ± 0.78	1.98 ± 0.70	0.0003
FEV1.0% (%)	72.4 ± 20.7	73.6 ± 10.8	0.82	70.2 ± 25.9	74.0 ± 12.6	0.67

[a] Lobectomy includes both lobectomy and wider resection.

FEV = forced expiratory volume.

and hemoptysis in 6. All patients had either a cavitary or a bronchiectatic lesion on chest computed tomography (CT). The lesions were predominantly cavities in 8 and predominantly bronchiectasis in 14 patients. In 9 women, the lesions were in the middle lobe or the lingular segment, that is, the so-called middle lobe or lingular type.

The MAC infections were confirmed in all patients from cultures or MAC-polymerase chain reaction methods performed in sputum or bronchoalveolar lavage fluid before surgery, according to the criteria of the American Thoracic Society [3]. All patients had received preoperative medications for 2 to 37 months (mean, 17). Clarithromycin was administered in 18 patients, combined with a new quinolone agent such as levofloxacin, ofloxacin or sparfloxacin in 9, and with rifampicin and ethambutol in 6 patient. Single-drug therapy was performed in 3 patients because they refused combined drug therapy because of adverse effects. In 9 recent cases, drugs were selected according to the results of microdilution antimycobacterial susceptibility test.

The performance status at the time of surgery was 0 in 19 patients and 1 in 3 patients.

Medical Therapy in Our Hospital

The outline of MAC medical therapy at the outpatient clinic of internal medicine in the recent 5-year period is as follows. Patients who were found at health screenings without symptoms were followed up for 3 to 6 months at the outpatient clinic. If the findings on chest roentgenogram worsened during this period, chemotherapy was started even in asymptomatic patients. Twenty-five patients with primary MAC disease were treated in our hospital during this 5-year period. Two patients had cavitary lesions, and other patients had bronchiectatic lesions or granular shadows. Each patient was treated by rifampicin, ethambutol, clarithromycin, and levofloxacin basically. The findings of sputum culture converted from positive to negative in 20 patients within 6 months. In the other 5 patients, the sputum culture remained positive, and consequently, these 5 patients underwent pulmonary resection. (Note: 5 of these 22 patients who underwent surgery are included in this study. The other patients in this study were referred to us from other facilities after receiving initial medical therapy.)

Surgical Procedures and Pathology

Bilateral lung resections were performed in 3 patients, including sequential bilateral resections within 1 day in 1 patient (no. 4). Resections of multiple lung regions were performed in 10 patients. The primary surgical procedures consisted of lobectomy in 14, bilobectomies in 1, segmentectomy in 4, and wedge resection of the lung in 6 patients. All wedge resections were performed using stapling devices. In 1 patient with lung cancer, the tumor was excised by left lower lobectomy, and partial resection of the upper lobe was performed for MAC. Wedge resection was performed by thoracoscopy using three ports in 3 patients. Lobectomy was performed assisted by thoracoscopy in 1 patient. We limited the thoracoscopic procedures to cases with small lesions because direct palpation is important to secure enough surgical margin in MAC patients. Thoracoplasty was performed in 1 patient (no. 11), with right upper lobectomy, because of insufficient expansion of remaining middle and lower lobes and existence of large postresectional pleural space.

Microscopic findings showed granulomatous inflammation with necrosis in all cases. In all surgical specimens, MAC was confirmed by microbiological methods including polymerase chain reaction.

Postoperative Follow-Up

Patients after surgery received follow-up every 3 or 4 months. Chest roentgenographic finding was checked in every patient. Smears, cultures, and polymerase chain reaction examinations of sputum were performed in patients with sputum. There was no postoperative mortality or major complication. One patient needed home oxygen therapy soon after discharge, but it was discontinued 2 months after surgery. All patients were alive 6 to 164 months after surgery. The median survival was 46 months. The results of postoperative pulmonary function testing, which was performed at more than 6 months after surgery (range, 6 to 156 months; median, 52), were available in 18 patients and are shown together with the preoperative data in Table 2. Both vital capacity and forced expiratory volume in 1 second were reduced significantly after resection. Both values, however, were maintained at 89% and 84% of preoperative values, respectively. The results were not significantly different

between patients who received lobectomy or wider resection (n = 11). The performance status after surgery was unchanged in all patients. The nutritional status of these patients was good and was not different before and after surgery.

Postoperative chemotherapy was administered to all patients. Positive sputum finding was observed in 2 patients after resection but became negative after postoperative chemotherapy. In 1 patient (no. 11), MAC-positive sputum detected after surgery became negative 4 months later while on chemotherapy. In another patient (no. 20), contralateral MAC lesions regressed after the resection of a large cavitary lesion.

Comment

The management of MAC pulmonary infection has made progress since the introduction of oral macrolides, and quinolones antibiotics. However, recent reports indicate that the medical treatment of MAC remains challenging. The conversion rates of sputum cultures from positive to negative with clarithromycin-containing regimens has been reported to be between 54% and 87% [4–8]. Moreover, reported recurrence rates after conversion range between 20% and 44% [4, 5, 7, 8]. Therefore we performed this retrospective study to evaluate the role of surgical resection after chemotherapy containing macrolides and quinolones.

Our main surgical indication is the persistence of localized lesions despite administration of multiple drugs listed earlier. Despite the administration of clarithromycin for 6 to 37 months in 18 patients who had cavitary or bronchiectatic lesions, the MAC lesions had persisted; and these organized MAC-infected lesions were uncontrollable by drug therapy alone, including clarithromycin. As recommended by several others [9, 10], we proceeded with surgical treatment early, when medical treatment appeared ineffective, before the lesions had become inoperable. In all our patients, the findings of MAC in the sputum became negative after surgery. Compared with reported postoperative conversion rates between 87% and 100% [9–13], our favorable results may attributable to the early performance of surgery.

Reports of long-term results of medical treatment for MAC pulmonary disease are very few. In the study of Kobashi and colleagues [14], the response rate of 115 patients treated according to proposed guidelines (rifampicin plus ethambutol plus streptomycin plus clarithromycin) was significantly better than that before the guidelines were established in primary MAC disease. However, poor outcomes, namely, "worsening" and "death," were still high in secondary MAC disease, at 23.1% and 10.3%. Although our follow-up is shorter, complete resection appeared to improve prognosis.

The MAC diseases were found at health maintenance examination in 10 asymptomatic patients of 22 (45%) in this series. The other 12 patients had mild symptoms. Because we performed surgery at an early stage of the disease, the rates of wedge or segmental resections were higher than reported by others. None of our patients required a pneumonectomy, and none suffered a major postoperative complication. Reported rates of major complications, such as bronchopleural fistulae and respiratory failure, associated with more invasive surgery, have been 0% to 42% [9–13]. Therefore, it appears important to avoid invasive surgery, such as pneumonectomy, if possible. Both vital capacity and forced expiratory volume in 1 second after resection were reduced slightly in this series. The performance status after surgery, however, was maintained even in lobectomy cases.

Our surgical procedures did not routinely include thoracoplasty, even after upper lobectomy, which was performed in 4 of our patients. We performed thoracoplasty in a single patient, who presented with a huge residual pleural space. Several authors [10, 13] have recommended muscle flaps to buttress the bronchial stump, and obliterate the empty space after pneumonectomy.

We performed three thoracoscopic wedge resections in this series of patients. Although it is a less invasive and technically easier procedure, resections of bronchiectatic lesions with secure surgical margins are difficult. Wedge resection was performed mainly for small cavitary lesion keeping enough margin along the bronchial wall with palpation in this series. Therefore, we limited our thoracoscopic wedge resections to one side and performed minithoracotomies on the other side in 1 patient (no. 4).

We observed 2 noteworthy patients, in whom contralateral lesions improved without organizing pulmonary changes, after resection of large cavitary lesions. We hypothesize that the resection of major bacterial foci may facilitate the chemotherapeutic management of lesions not associated with cavities or bronchiectasis.

In conclusion, we recommend that surgical treatment of MAC pulmonary lesions be performed before the disease becomes too advanced, and difficult to resect. Patients treated with a clarithromycin-containing regimen for more than 6 months for MAC pulmonary disease with cavitary or bronchiectatic lesions, even when asymptomatic, should be viewed as candidates for surgery, as it is associated with low morbidity and mortality. In extensive disease, after the resection of major cavitary bacterial foci, the contralateral lesions can be further managed postoperatively by chemotherapy.

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特　集

縦隔疾患に対する外科的アプローチ

3．胸腺上皮性腫瘍の外科治療成績

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キーワード　胸腺腫，胸腺癌，正岡分類，WHO 分類，外科治療

I．内容要旨

胸腺腫を含む胸腺上皮性腫瘍は頻度の多い腫瘍ではない．大半の胸腺種は外科治療によって良好な予後が得られるものの，術後の再発，進展を呈する症例では治療に難渋することもある．当科にて1985年より2005年までの胸腺関連腫瘍のうち組織学的に胸腺腫あるいは胸腺癌と診断された症例で，予後を追跡でき，病理標本をWHO分類に当てはめて再検討した131例を対象として外科治療成績を検討した．男性76例，女性55例，平均年齢53歳（20歳から80歳）正岡の分類ではI期42例，II期43例，III期23例，IVa期15例，IVb期1例，胸腺癌（扁平上皮癌）7例であった．WHO分類ではType A 7例，Type AB 23例，Type B1 30例，Type B2 27例，Type B3 29例，Type C 15例であった．実施手術は生検5例，腫瘍切除5例，胸腺摘出5例，拡大胸腺全摘術が65例，腫瘍切除に合併切除を行った症例が4例，拡大胸腺全摘術に合併切除を行った症例が51例であった．合併切除の内訳は播種巣1例，胸膜合併切除14例，心膜合併切除が10例，肺合併切除が4例，2種類以上の組織，器官，臓器を合併切除した症例が22例であった．Masaokaの分類を用いて予後を検討したところ，5年，10年，15年生存率は，I期で100%，100%，100%，II期で100%，100%，87.5%，III期で100%，87.5%，87.5%，IVa期で71.1%，53.3%，53.3%，胸腺癌症例では42.9%，42.9%，0%であった．IVa期および胸腺癌の予後は他の病期と比較して有意に予後が不良であったが，I期，II期，III期の間には生存には有意差を認めなかった．WHO分類では，Type Aでは5年生存率100%，Type ABでは5年，10年，15年生存率が100%，100%，100%，Type B1では100%，100%，75.0%，Type B2では92.6%，86.4%，86.4%，Type B3では95.5%，95.5%，81.8%Type Cでは57.1%，42.9%，0%であった．Type Cの予後はType B3と比較すると予後の悪い傾向が認められた．Type Cとその他の群との比較ではType Cの予後が有意に不良であった．胸腺上皮系腫瘍の外科治療成績は胸腺癌も含め，病理組織学的性格と腫瘍の浸潤の程度によって決定されると考えられた．胸腺上皮系腫瘍の内でも浸潤型胸腺腫（Type B3）および胸腺癌（Type C）の治療成績は良好とは言えず，生検による病理組織学的検討と画像診断による浸潤傾向の診断を行って集学的治療を行うことが不可欠であると考えられた．

II．はじめに

胸腺腫を含む胸腺上皮性腫瘍は頻度の多い腫瘍ではないが，進展形式と合併疾患の多様性など特徴的な性格を有する．大半の胸腺種は外科治療によって良好な予後が得られるものの，浸潤傾向が強かったり，縦隔外への進展を呈する症例では手術後遠隔期に再発を起こし，治療に難渋する症例もある．胸腺腫の外科治療成績を評価する際に基準となる評価方法は1940年代

PROGNOSIS OF SURGICALLY TREATED THYMIC EPITHELIAL TUMORS
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表1 胸腺腫症例の分布 Characteristics of assessable patients

	No of patients	Age	Gender (M : F)	P	Myasthenia Gravis (+ : −)	P	Median follow up (Month)
Masaoka							
I	42	54 (22-76)	27 : 15	P = 0.62	8 : 34	p = 0.06	81 (30-232)
II	43	52 (22-75)	23 : 21		20 : 24		85 (10-195)
III	23	53 (21-80)	17 : 11		8 : 20		57 (6-202)
IVa	15	54 (40-76)	8 : 8		6 : 10		48 (0-177)
IVb	1	66	1 : 0		0 : 1		1
Ca	7	62 (52-76)	6 : 1		1 : 6		57 (34-177)
WHO							
A	7	54 (49-68)	5 : 2	P = 0.21	0 : 7	p = 0.02	59 (2-90)
AB	23	48 (25-72)	13 : 10		6 : 17		62 (14-232)
B1	30	53 (22-76)	15 : 15		10 : 20		93 (6-219)
B2	27	52 (21-80)	18 : 9		12 : 15		99 (3-207)
B3	29	52 (40-75)	13 : 16		14 : 15		86 (12-177)
C	15	61 (30-78)	12 : 3		1 : 14		54 (0-118)

より模索されてきた．現在まで予後を決定する因子として，正岡の分類[1]，WHO分類[2][3]，TNM分類[4]，Grupped' Etude des Tumeurs Thymiques system[5]などが発表され，実際に臨床で用いられているが，最も予後を反映するものは正岡の分類とWHO分類であると考えられている．

正岡の分類は手術所見および組織学的な浸潤の有無による分類であり，組織学的な悪性度評価との関係が明らかでないことと，術前での評価が困難であることが欠点として挙げられていた．1985年にMarinoとMullar-Hemerinkにより胸腺腫を組織学的に分類することにより予後と相関するとの報告があり[6]，その後胸腺腫を組織学的特徴によって6つの組織型に分類するWHO分類が1999年に発表されることとなった[3]．以後，WHO分類が胸腺腫の臨床像を反映するかについて検討され，予後との相関のみならず，術前治療の指標となるとの報告がなされている[7]~[9]．今回当科で経験した胸腺腫と胸腺癌を含む胸腺上皮性腫瘍症例の外科治療成績を検討するにあたり，Retrospectiveに病理の評価を行い，臨床情報とともに検討した．

III. 対　象

当科にて1985年より2005年までの胸腺関連腫瘍のうち組織学的に胸腺腫あるいは胸腺癌と診断された症例で，予後を追跡でき，病理標本をWHO分類に当てはめて再検討した131例を対象とした．

男性76例，女性55例，平均年齢53歳（20歳から80歳）正岡の分類ではI期42例，II期43例，III期23例，IVa期15例，IVb期1例，胸腺癌（扁平上皮癌）7例であった．WHO分類ではType A 7例，Type AB 23例，Type B1 30例，Type B2 27例，Type B3 29例，Type C 15例であった．

実施した手術は生検のみ5例，腫瘍切除5例，胸腺摘出5例，拡大胸腺全摘術が65例，腫瘍切除に合併切除を行った症例が4例，拡大胸腺全摘術に合併切除を行った症例が51例であった．合併切除の内訳は播種巣1例，胸膜合併切除14例，心膜合併切除が10例，肺合併切除が4例，2種類以上の組織，器官，臓器を合併切除した症例が22例であった．

重症筋無力症の有無では重症筋無力症の合併を認めた症例が43例，合併のない症例が88例であった．

予後をKaplan-Meier法によって生存曲線を作成し，Log rank法にて群間の有意差を検討した．各群における独立性の検定にはχ二乗検定を用いた．$p \leqq 0.05$で有意差ありと判定した．

IV. 結　果

群別に独立性を検定したところ，正岡の分類では，年齢，性別，MGの有無の項目は独立した事象であった．WHO分類では年齢，性別は独立事象と考えられたが，Type AではMG症例を認めず，WHO分類による組織型とMGの間に相関がある結果となったが，Type Aを除いて検討すると有意差はなく，MGと組織型は独立事象であった．

Masaokaの分類を用いて予後を検討したところ，5

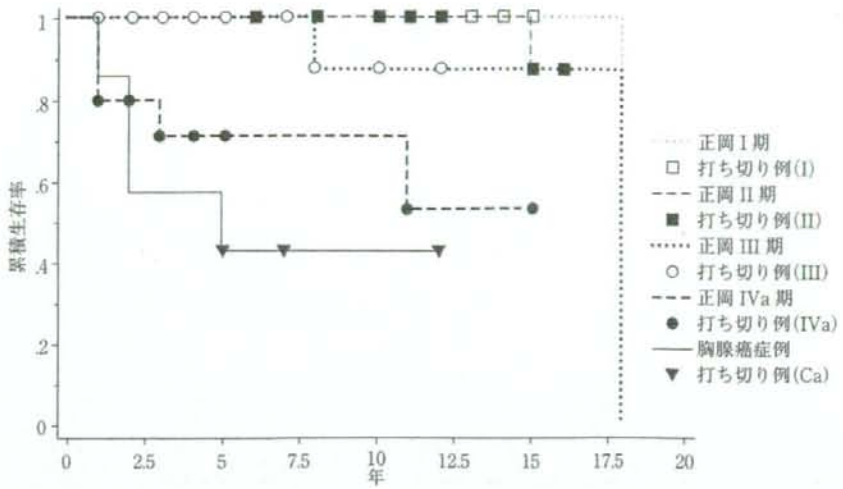

図1　正岡分類による予後（含胸腺癌）Kaplan-Meier 法

5, 10, 15year survival in each group are 100%, 100%, 100% in Stage I, 100%, 100%, 87.5% in Stage II, 100%, 87.5%, 87.5% in Stage III, 71.1%, 53.3%, 53.3% in Stage IVa, 42.9%, 42.9%, 0% in Thymic cancer respectively. Survival of IVa and thymic carcinoma are significantly poor compare to Stage I, II, and III.

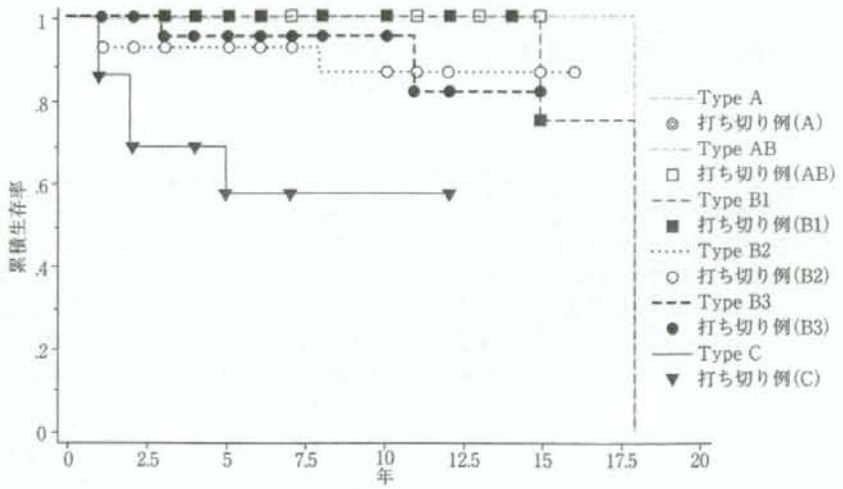

図2　WHO 分類による予後 Kaplan-Meier 法

According to WHO classification, 5 year survival of type A was 100%, 5, 10, 15 year survival are 100%, 100%, 100%in type AB, 100%, 100%, 75.0% in type B1, 92.6%, 86.4%, 86.4% in type B2, 95.5%, 95.5%, 81.8% in type B3, 57.1%, 42.9%, 0% in type C. Survival of Type C was the poorest among all and significant difference between al other groups were recognized.

年，10 年，15 年生存率は，I 期で 100%，100%，100%，II 期で 100%，100%，87.5%，III 期で 100%，87.5%，87.5%，IVa 期で 71.1%，53.3%，53.3%，胸腺癌症例では 42.9%，42.9%，0% であった（図 1）．IVa 期お

よび胸腺癌の予後は他の病期と比較して有意に予後が不良であったが，I期，II期，III期の間には生存には有意差を認めなかった（図1）．

WHO分類を用いて予後を検討したところ，Type Aでは5年生存率100%，Type ABでは5年，10年，15年生存率が100%，100%，100%，Type B1では100%，100%，75.0%，Type B2では92.6%，86.4%，86.4%，Type B3では95.5%，95.5%，81.8%Type Cでは57.1%，42.9%，0%であった（図2）．Type Cの予後はType B3と比較するとp=0.053で予後の悪い傾向が認められた．Type Cとその他の群との比較では各群間と比較してType Cの予後が有意に不良であった（図2）．

V. 考　察

胸腺上皮系腫瘍（胸腺腫および胸腺癌）の臨床的悪性度（再発と予後）については組織学的所見と乖離があることが述べられていた[5][10][11]．1984年に発表された正岡の分類は手術所見（浸潤の程度）によって悪性度を判断する分類[1]であるが，予後と無再発生存を良く反映するので現在でもひろく使用されている[1][12]．しかし，正岡の分類は基本的に手術所見による浸潤傾向を臨床的に判断するもので，術前に悪性度を評価することが困難であった．

胸腺上皮腫瘍の組織学的分類はBernatzらのリンパ球の混在度と上皮性分の細胞形態から判断する分類が1961年に発表され[13]，これに準じる分類が採用されることが多かったが，予後との関連に乏しいといわれていた[11]．Nomoriらは核面積による予後の解析を行い[14]，予後との関連を報告したが，臨床に採用されるまでには至らなかった．MarinoとMueller-Hermelinkは1985年に腫瘍細胞と胸腺の皮質および髄質における胸腺上皮との類似性を基礎とした組織学的分類を発表していた[6]．この分類のなかでWell differentiated carcinoma（WDC）と呼ばれるカテゴリーにおいては単一の腫瘍細胞の増生だけでなく，リンパ球の混在があってもよいとし，胸腺に発生する扁平上皮癌などと比較すると予後が良いことが指摘されていた[15]．WHOは胸腺上皮腫瘍の分類を行うに当たり，各国の病理学者を集めたパネル協議を通じて1999年に基本的にMueller-Hermelinkらの分類に準じた分類を決定して発表した[3]．それによると，Type Aは核異型のない紡錘形から卵円形の上皮細胞からなり，腫瘍性格を持つリンパ球がほとんどない腫瘍，Type ABはリンパ球に富んだ部位を混じるが基本的には腫瘍細胞がType Aに類似した上皮形態を取る腫瘍，Type B1は正常な機能胸腺の皮質あるいは髄質に存在する胸腺上皮に類似した腫瘍細胞からなる腫瘍，Type B2はリンパ球が多く混在し，空胞化した核とはっきりした核小体を有する腫瘍細胞が散在性認められ，perivascular spaceをよく認めるもの，Type B3は円形から多角形の腫瘍細胞によって占められ，核異型は軽度で，リンパ球の混在の少ないもので，扁平上皮化成やperivascular spaceが通常認められる腫瘍，Type Cは細胞の異型が強く他の臓器の腫瘍で癌と診断すべきもので，リンパ球が混在しても，成熟型のリンパ球や形質細胞であるものとしている．

当科で1985年から2005年までに経験しRetrospectiveにWHO分類による判定を行った胸腺上皮腫瘍131例の予後を解析した．正岡の分類の中には含まれない扁平上皮癌症例を胸腺癌としてあわせて検討した．胸腺癌症例は5年生存で42.9%と不良であった．IVa期症例も5年生存71.1%と早期に再発による死亡が認められた．II期，III期の症例では，予後は良好とはいえ，死亡症例が認められたが，I期症例では良好な予後が得られていた．

WHO分類により予後を検討したところ，Type Cでは5年生存57.1%と不良であった．Type Cの成績は他の群との間に有意差を認めた．他の群の5年生存率はType A 100%，Type AB 100%，Type B1 100%，Type B2 92.6%，Type B3 95.5%とこれらの群間には予後に有意差を認めていない．Okumuraら[7]，Nakagawaら[16]の報告では20年以上の観察期間で検討してType B1とType B2，Type B2とType B3の間に有意差を認めている．長期の観察を必要とする胸腺腫の生物学的特徴によるものと考えられる．術後の補助療法の有無が予後に影響を与えている可能性もあると思われる．WHO分類による胸腺腫の予後の検討はMineo[8]，Rena[17]らによっても行われ，この分類が腫瘍の悪性度を反映すると報告しているが，いずれの報告でも正岡の病期分類は独立した予後決定因子であることを明らかにしている．

以上の結果から，生存に関しては臨床所見を重視した正岡の分類によるほうが予後を明確に弁別できると考えられた．Wrightらは胸腺腫の再発を予測するのに浸潤の程度，WHO分類と腫瘍径が重要であると報告しており，正岡分類も再発に関与する独立した因子であるとしている[9]．病理学的分類と臨床所見の立場

からの病期分類を加味して診療に役立てることが重要であると考えられる.

外科治療成績の悪い群では集学的治療を考慮すべきであり，当科では画像診断によって浸潤の有無を判断し，症例を選択して術前化学療法や術後照射を行っている.

当科の症例では，正岡分類のII期の症例では43例中8例に術後照射が行われ，III期症例では23例中18例に術後照射，3例に化学療法が行われ，IVa期症例では15例中10例に術前化学療法を1例に術後化学療法を，照射を12例に行っている．術前に化学療法を行うことで大血管や肺に浸潤する胸腺腫症例において腫瘍の縮小が得られ，合併切除の範囲を縮小することができるため，画像診断で浸潤臓器が明らかな場合には術前化学療法を施行したほうがよいと考えられる．胸腺腫に対する照射の効果については加勢田[18]やUematsuら[19]が少量の全肺照射を含む放射線治療を行うと，照射野内での再発が抑制されるとの報告を行っている．これらの補助療法が本検討の予後を改善している可能性があると考えられる.

また，重症筋無力症合併症例に関しては重症筋無力症の消長と腫瘍の再発の関係が不明であり，術後遠隔期に重症筋無力症のみならず，赤芽球癆や無ガンマグロブリン血症を発症する症例も経験されることなどから今回は検討しなかった．重症筋無力症の合併については予後が不良となるとの報告[20]と，予後に関係しないとの報告[7]がある．最近の多変量解析の結果では予後に影響を与えないと考えられている[7].

VI. おわりに

胸腺上皮系腫瘍の外科治療成績は胸腺癌も含め，病理組織学的性格と腫瘍の浸潤傾向によって決定されると考えられた．胸腺上皮系腫瘍の内でも浸潤型胸腺腫および胸腺癌の治療成績は良好とは言えず，この成績を改善するためには集学的治療が不可欠で，生検による病理組織学的検討と画像診断による浸潤傾向の診断を行って術前治療の必要性を判断することが重要であると考えられる.

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PROGNOSIS OF SURGICALLY TREATED THYMIC EPITHELIAL TUMORS

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This study was performed to clarify the prognosis of patients with surgically treated thymic epithelial tumors. The records of 131 patients who underwent surgical treatment during 1985-2005 were retrospectively reviewed. Pathologic review was done according to the WHO classification of tumors of the thymus. Patients characteristics were : 76male and 55fimale ; average age 53 (range 20-80) years ; tumor stage was stage I in 42, stage II in 43, stage III in 23, stage IVa in 15, stage IVb in 1, and thymic carcinoma (squamous cell carcinoma) in 7 based on Masaoka's staging. There were 7 cases of type A, 23 of type AB, 30 of type B1, 27 of type B2, 29 of type B3, and 15 of type C. Surgical procedures performed were 5 partial resections, 5 tumoretomies, 5 thymectomies, 65 extended thymectomies, 4 tumorectomies plus adjunctive resections of surrounding tissue, and 51 extended thymectomies plus tumorectomies plus adjunctive resections of surrounding tissue including the pleura, pericardium, lung, and great vessels. Five-, 10-, and 15-year survival rates by Masaoka stage were 100%, 100%, and 100% in stage I ; 100%, 100%, and 87.5% in stage II ; 100%, 87.5%, and 87.5% in stage III ; 71.1%, 53.3%, and 53.3% in stage IVa ; and 42.9%, 42.9%, and 0% in thymic carcinoma. The prognosis of patients with stage IVa and thymic carcinoma was thus significantly poorer compared with that in the other groups. According to the WHO classification, the 5- year survival rate of type A was 100%, and the 5-, 10-, and 15-year survival rates were 100%, 100%, and 100% in type AB ; 100%, 100%, and 75.0% in type B1 ; 92.6%, 86.4%, and 86.4% in type B2 ; 95.5%, 95.5%, and 81.8% in type B3 ; and 57.1%, 42.9%, and 0% in type C. The survival rate of patients with type C was the poorest and there was a significant difference between type C and all other groups.

The prognosis of patients with thymic epithelial tumors after resection is thought to be determined by histologic classification and clinical invasiveness. In particular, patients with type B3 and type C thymomas should be considered for multidisciplinary treatment.

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Anti-High-Mobility Group Box Chromosomal Protein 1 Antibodies Improve Survival of Rats with Sepsis

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Abstract

Background: High-mobility group box chromosomal protein 1 (HMGB1) has recently been shown to be an important late mediator of endotoxin shock, intraabdominal sepsis, and acute lung injury, and a promising therapeutic target of severe sepsis. We sought to investigate the effect of antibodies to HMGB1 on severe sepsis in a rat cecal ligation and puncture (CLP) model.

Methods: Adult male Sprague-Dawley rats underwent CLP and then were randomly divided into two groups: treatment with anti-HMGB1 polyclonal antibodies, and non-immune IgG-treated controls. The serum HMGB1 concentrations were measured at ten time points (preoperatively, and postoperatively at 4, 8, 20, 32, and 48 h and at 3, 4, 5, and 6 days). Hematoxylin-eosin staining, elastica-Masson staining, and immunohistochemical staining for HMGB1 were performed on the cecum and the lung to assess pathological changes 24 h after the CLP procedure.

Results: Treatment with anti-HMGB1 antibodies significantly increased survival [55% (anti-HMGB1) vs. 9% (controls); $P < 0.01$]. The serum HMGB1 concentrations at postoperative hours

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20 and 32 of the anti-HMGB1 antibody-treated animals were significantly lower than those of the controls ($P < 0.05$). Treatment with anti-HMGB1 antibodies markedly diminished the pathological changes and the number of HMGB1-positive cells in the cecum and the lung.
Conclusions: The present study demonstrates that anti-HMGB1 antibodies are effective in the treatment of severe sepsis in a rat model, thereby supporting the relevance of HMGB1 eradication therapy for severe sepsis.

High-mobility group box chromosomal protein 1 (HMGB1) is an abundant, highly conserved cellular protein that binds to DNA and stabilizes nucleosome formation, facilitates gene transcription, and regulates the activity of steroid hormone receptors.[1–4] It has recently been shown to be a crucial late mediator of endotoxin shock, fecal peritonitis, and acute lung injury, and is a promising therapeutic target of severe sepsis.[1–4]

Cecal ligation and puncture (CLP) is a commonly used experimental animal model of sepsis.[5–7] This model most closely resembles bowel perforation, with tissue necrosis leading to polymicrobial infection similar to the scenario of acute peritonitis. CLP in rodents results in an early hyperdynamic phase which is followed by a hypodynamic phase, as in human sepsis.[8] Because HMGB1 is actively released into the serum by activated monocyte/macrophages and passively diffuses from necrotic cells, CLP animals have two possible sources of HMGB1: monocyte/macrophages activated by bacterial peritonitis, and necrotic cecum.[9–11]

In 2004, Yang *et al.*[12] reported that—in a murine CLP model—treatment with anti-HMGB1 antibodies and imipenem after CLP surgery significantly increased survival. In the present study, we have modified the conventional CLP model by preserving the feeding artery and the drainage vein. In this model, severe bacterial peritonitis was observed without necrosis of the ligated cecum. We hypothesized that anti-HMGB1 antibodies alone would be effective in modified CLP sepsis and that anti-HMGB1 antibodies would be effective in animals other than mice. The present study was therefore designed to assess whether anti-HMGB1 antibodies alone would have protective effects in a clinically relevant rat sepsis model without necrosis of the cecum.

MATERIALS AND METHODS

Animals

Male 8-week-old specific pathogen-free Sprague-Dawley rats, each weighing 250–300 g, were purchased from CLEA Japan (Tokyo, Japan). The animals were allowed to acclimate for 7 days before use in the Laboratory Animals Center, Keio University School of Medicine under standard temperature and light and dark cycles. The rats had access to chow and water ad libitum throughout the study. All procedures were performed with the approval of the Laboratory Animal Care and Use Committee at Keio University School of Medicine.

Cecal Ligation and Puncture

To establish live intraabdominal infection and sepsis, we subjected the rats to the CLP procedure.[5,6] The animals were first anesthetized by intramuscular injection of ketamine (40 mg/kg of body weight), and a 20-mm midline incision was made to expose the cecum. The cecum was mobilized, ligated below the ileocecal valve while preserving the blood flow of the cecum, then a 5.0-mm blade incision was made at the tip of the cecum. The cecum was replaced in its normal intraabdominal position and the wound closed with a running suture. All animals received saline-solution (0.9% subcutaneously, 5.0 ml/kg of body weight) resuscitation immediately after the surgery.

Antibody Production

Isolation of HMGB1 from porcine thymus chromatin

Porcine HMGB1 was obtained from porcine thymus. HMGB1 was extracted with 0.75 *M* $HClO_4$ and by fractioned precipitation with acetone. Samples were purified by ion-exchange chromatography, (CM-sephadexC25; Pharmacia, Uppsala, Sweden) and analyzed by polyacrylamide gel electrophoresis.

Production of anti-HMGB1 polyclonal antibody and control IgG antibody

Porcine HMBG1 was used as immunogen. Hens were injected with porcine HMGB1, and IgG was purified from egg yolk.[13,14] The titration of the antibodies purified was carried out by enzyme-linked immunosorbent assay (ELISA). These anti-HMGB1 polyclonal antibodies were

prepared as previously described.[14] Control IgG antibodies were purified from non-immunized egg yolk.

Experimental Design

After CLP was performed on Sprague-Dawley rats, they were subcutaneously injected with anti-HMGB1 antibodies (10 mg/kg of body weight) or control antibodies (10 mg/kg of body weight) in a total volume of 500 μl of sterile saline 15 min after CLP surgery. The dosage of the anti-HMGB1 antibodies was determined based on the least amount that could completely neutralize 3 ng/mL of serum HMGB1 (*i.e.*, the maximal serum HMGB1 concentrations of the control animals) for 24 h *in vitro* (unpublished data). The dosage of the control antibodies was determined to be the same as that of the anti-HMGB1 antibodies. Each group contained 11 animals. Survival was monitored every 4 h for the subsequent 10 days. All of the animals were dissected at death or sacrifice, and intraabdominal and intrathoracic findings were recorded. The surviving animals were killed with diethyl ether on postoperative day (POD) 10.

Measurements

Blood samples (0.6 mL) were collected from the jugular vein under anesthesia by intramuscular injection of ketamine (40 mg/kg of body weight), centrifuged, and stored at −80°C. The serum HMGB1 concentrations were measured at ten time points [preoperatively, and postoperatively at hours (POH) 4, 8, 20, 32, 48, and at days (POD) 3, 4, 5, and 6 by enzyme-linked immunosorbent assay (ELISA; Central Institute, Shino-Test, Kanagawa, Japan)].[15]

Histological Evaluations

Cecal ligation and puncture and antibody treatment were performed on two groups of three Sprague-Dawley rats in the same manner as described above. The surviving animals were killed with diethyl ether 24 h after the surgery, and the cecal tissues and the pulmonary tissues were removed from the animals and processed for histologic studies. Hematoxylin-eosin (H&E), elastica-Masson (EM), and immunohistochemical staining for HMGB1 were performed.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues from rat specimens were cut into 4-μm sections. Each section was mounted on a silane-coated glass slide, deparaffinized, and soaked for 15 min at room temperature in 0.3% H_2O_2/methanol to block endogenous peroxidase. Trypsin (Nichirei, Tokyo, Japan) treatment for 10 min at room temperature was performed for antigen retrieval. A murine anti-HMGB1 monoclonal antibody (Central Institute, Shino-Test) was applied for 12 h at 4°C. The primary antibody was visualized using the Histofine Simple Stain MAX-PO(M) kit (Nichirei, Japan) according to the instruction manual.[16] The slide was counterstained with hematoxylin.

Statistical Analysis

Survival at 10 days was compared by Kaplan-Meier analysis and by log rank statistics, with a P value of less than 0.05 considered to indicate a statistically significant difference. To identify the impact of treatment with anti-HMGB1 antibodies on the time course of changes in serum HMGB1 concentration, we compared the serum HMGB1 concentrations of the animals treated with anti-HMGB1 antibodies (n = 11) and controls (n = 11) at every time point. The comparisons between the data were made with a non-parametric Mann-Whitney U-test, with a P value of less than 0.05 considered to indicate a statistically significant difference. To assess the efficacy of treatment with anti-HMGB1 antibodies, an analysis was also performed with the "last observation carried forward" method.[17] The statistical analyses were performed with the StatView 5.0 and SAS statistical analysis package (SAS Institute, Cary, N.C.).

RESULTS

Survival Rates

Of the 11 animals treated with anti-HMGB1 antibodies, six survived for 10 days, but only one of the animals treated with control antibodies survived for that same period. A significant difference between the two groups was seen when the whole period of follow up was compared with Kaplan-Meier analysis and log rank test ($P < 0.01$) (Figure 1).

Time Course of Changes in Serum HMGB-1 Concentrations

Serum HMGB1 concentrations of the controls increased during the first 20 h postoperatively and remained higher than 2 ng/ml thereafter. In contrast, serum HMGB1 concentrations of the animals treated with anti-

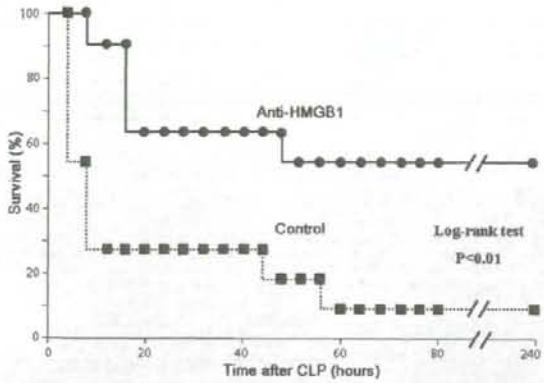

Figure 1. Treatment with anti-HMGB1 antibodies improved survival in CLP rats. Survival was analyzed in Sprague-Dawley (SD) rats subjected to CLP, and the treatment with anti-HMGB1 antibodies was started 15 min after CLP surgery. Data are shown as percentages of animals surviving (n = 11 in each group). The whole period of follow up is compared with a Kaplan-Meier analysis and log rank test ($P < 0.01$). ●, anti-HMGB1 antibody-treated group; ■, controls.

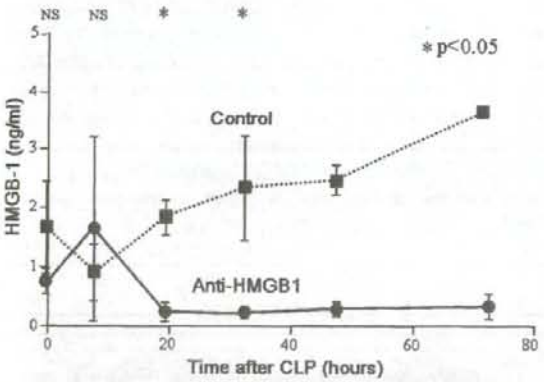

Figure 2. Serum HMGB1 concentrations of the animals treated with anti-HMGB1 antibodies were significantly lower than those in the controls at POH 20 and POH 32. Data are shown as means ± SEM of 11 rats in each group. The comparisons between the data at every time point were made with a non-parametric Mann Whitney *U*-test, with *P* values < 0.05 considered to indicate a statistically significant difference. ●, anti-HMGB1 antibody-treated group; ■, controls.

HMGB1 antibodies were significantly lower than in the controls at POH 20 and POH 32, and they remained lower than 0.3 ng/ml (Figure 2).

At POH 4, the serum HMGB1 concentration in the controls had markedly increased ($n = 11$; median: 2.7 ng/ml; interquartile range: 0–11.5 ng/ml), while it had increased to a much lesser extent in the anti-HMGB1 antibody-treated animals ($n = 11$; median: 0.2 ng/ml; interquartile range: 0–0.55 ng/ml); however, this difference was not statistically significant. Of note, serum HMGB1 concentrations in control animals who died by POH 4 were remarkably high ($n = 5$; median: 18.5 ng/ml; interquartile range: 3.6–82 ng/ml).

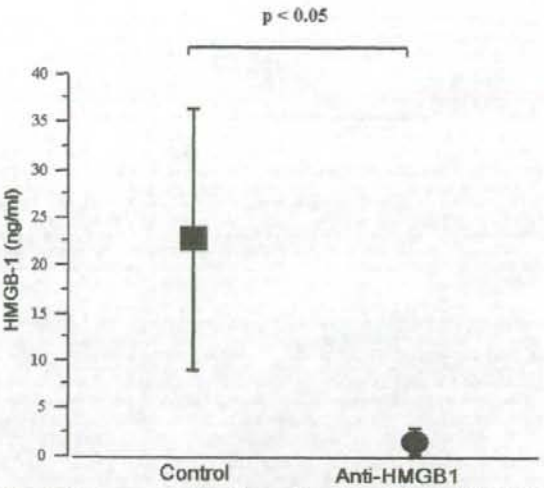

Figure 3. To assess the efficacy of treatment with anti-HMGB1 antibodies, we performed an analysis with the "last observation carried forward" method. The levels of the animals treated with anti-HMGB1 antibodies were found to be significantly lower than those of controls ($P < 0.05$ by a non-parametric Mann-Whitney *U*-test).

The kinetics of serum HMGB1 concentrations tended to correspond with the development of clinical signs of sepsis, such as piloerection, diminished activity, loss of exploratory behavior, and appetite loss. Although most controls developed these signs by POH 8 and succumbed by POD 2, anti-HMGB1 antibody-treated animals were observed to be more alert and active, and only one death occurred after POH 16, as shown in Figure 1.

At the time of death or sacrifice, serum HMGB1 levels in animals treated with anti-HMGB1 antibodies were significantly lower than those of controls ($P < 0.05$ by the "last observation carried forward" method) (Figure 3).

Intraabdominal and Intrathoracic Findings at the Time of Death or Sacrifice

In both the anti-HMGB1 and control groups, all the animals that died after CLP showed findings typical of diffuse peritonitis, including severe inflammation around the punctured cecum and a large amount of fecal and suppurative ascites. Macroscopic alveolar bleeding and

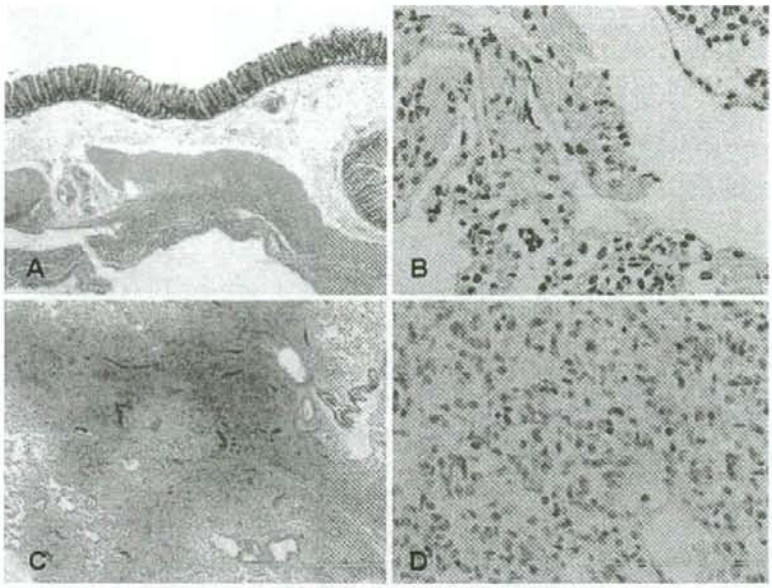

Figure 4. Histological and immunohistochemical findings in controls. **A.** H&E staining of the cecum (original magnification, 40×). **B.** Immunohistochemical staining of HMGB1 in the cecum (original magnification, 400×). **C.** Elastica-Masson staining of the lung (original magnification, 40×). **D.** Immunohistochemical staining of HMGB1 in the lung (original magnification, 400×).

pleural effusion were observed in the thoracic cavity of the controls, but such findings were not observed in the anti-HMGB1 antibody-treated animals. In contrast, in both groups, there were no deaths after POD 10. In the surviving animals, the tip of the cecum was covered with intestine and intraabdominal fat tissue, and no foci of acute inflammation or ascites were observed. No remarkable findings were observed in the thoracic cavity or the lungs of the animals who survived POD 10.

The cecums of all the animals both dead and sacrificed were not necrotic but atrophic. No necrosis was observed based on preserving the blood flow of the ileocolic artery and vein according to H&E staining of the cecum of a control at POD 10. No differences were detected between the cecum of the control surviving for 10 days and that of the anti-HMGB1 antibody-treated animals surviving for 10 days.

Histopathological and Immunohistochemical Findings of the Cecum and the Lung at POH 24

H&E staining of the cecum of the controls showed marked infiltration of inflammatory cells into the subserosa (Figure 4A). The cytoplasm of the erosive mucosa and both nuclei and cytoplasm of many of the inflammatory cells were positively stained for HMGB1 (Figure 4B). Destruction of the pulmonary cell walls, migration of inflammatory cells, and pulmonary hemorrhage were present in the lungs of controls (Figure 4C). In control animals, a large number of HMGB1-positive alveolar macrophage-like cells were present, as were epithelial cells with positive nuclear staining (Figure 4D).

In animals treated with anti-HMGB1 antibodies, H&E staining of the cecum and EM staining of the lung showed almost no destructive or inflammatory changes (Figure 5A, C). Much fewer HMGB1-positive cells were detected in the cecum and the lungs in the anti-HMGB1 antibody-treated group (Figure 5B, D).

DISCUSSION

Our study yielded two major findings. First, treatment with anti-HMGB1 antibodies after CLP prolonged survival in rats without any antibiotics. Second, serum HMGB1 levels of the controls dramatically increased as early as 4 h after CLP, even though HMGB1 has been considered to be a late mediator of sepsis.

In a previous study, we clarified that the surgical stress of transthoracic esophagectomy itself induced the increase in serum HMGB1, even in patients without complications, and that elevations in HMGB1 might play a contributory role in the development of postoperative organ system dysfunction.[18] Moreover, the patients with sepsis after esophagectomy showed significantly higher serum HMGB1 levels.[18] We proposed that because serum HMGB1 concentrations increased later than inflammatory cytokines, such as IL-1β, and IL-6, modu-

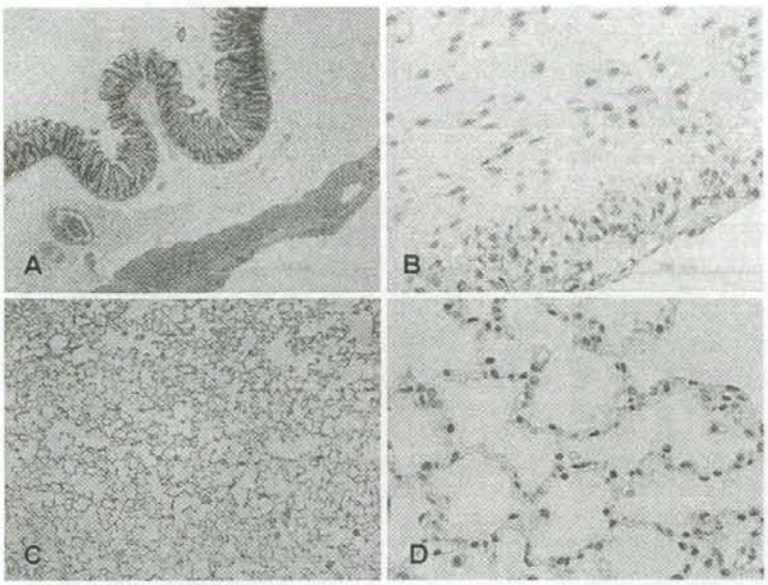

Figure 5. Histological and immunohistochemical findings in anti-HMGB1 antibody-treated group. **A.** H&E staining of the cecum (original magnification, 40×). **B.** Immunohistochemical staining of HMGB1 in the cecum (original magnification, 400×). **C.** Elastica-Masson staining of the lung (original magnification, 40×). **D.** Immunohistochemical staining of HMGB1 in the lung (original magnification, 400×).

lating serum HMGB1 concentrations would be more likely to have wider therapeutic windows than modulating serum inflammatory cytokines with the aim of effecting a decrease in surgical stress and an improvement in postoperative clinical courses.[18] With respect to cytokine modulation in esophagectomy, only preoperative administrations of corticosteroid, ulinastatin, and gabexate mesilate have been reported to be effective.[19–21] Working under the consideration that postoperative modulation of serum HMGB1 concentrations might be effective in improving postoperative clinical courses and preventing postoperative complications, we attempted to develop a modified CLP model without antibiotics as a model of systemic inflammatory response syndrom (SIRS)-related multiple organ dysfunction syndrome and to administer anti-HMGB1 antibodies to the model immediately after CLP surgery. In the present study, we developed a modified CLP rat model without necrosis of the cecum by preserving the blood flow of the ileocolic artery and vein. Serum HMGB1 concentrations of the control group rose within 8 h following surgery and were maintained at high concentrations for a period of time, and so we chose subcutaneous injection of anti-HMGB1 antibodies rather than intravenous injection. Because treatment with anti-HMGB1 antibodies alone after CLP significantly prolonged survival in rats without any antibiotics, it is possible to speculate that HMGB1 eradication therapy immediately after surgery may offer a clinically relevant prophylaxis or early intervention to surgical stress-induced organ dysfunction in high-risk cases.

In these experiments, we evaluated the therapeutic effects of anti-HMGB1 antibodies in terms of survival, serum HMGB1 concentrations, and histological changes in the cecum and the lungs. All deaths occurred early, and the marked difference in survival occurred prior to POH 20, actually at POH 12. Serum HMGB1 concentrations of the controls, especially in animals that died, dramatically increased by POH 4, whereas, serum HMGB1 concentrations in anti-HMGB1 antibody-treated animals were significantly suppressed at POH 20 and 32, but this suppression did not begin until POH 8. At POH 24, the inflammatory changes and HMGB1 expression in the cecum and the lungs of the anti-HMGB1 antibody-treated animals were much less than those of the controls. Taken together, these data demonstrate that: (1) anti-HMGB1 antibodies administered subcutaneously 15 min after CLP surgery started to neutralize local and serum HMGB1 between POH 4 and 8 and suppressed both the local inflammatory response as well as inflammation in remote organs, thereby resulting in an improvement in survival; (2) in anti-HMGB1 antibody-treated animals, neutralization of serum HMGB1 as well as suppression of both local and remote inflammation led to suppression of serum HMGB1 concentrations at POH 20 and 32. In the modified CLP model without antibiotics, serum HMGB1 concentrations elevate relatively earlier than in conventional models with sepsis.[1,3,12] Although statistically significant

differences between the anti-HMGB1 antibodies-treated group and the control were not found for serum HMGB1 concentrations at POH 4 because of the large deviation, serum HMGB1 concentrations did increase much earlier and higher in those animals destined to die than in those destined to survive. Moreover, treatment with anti-HMGB1 antibodies significantly prolonged survival. These results suggest that HMGB1 plays a critical role in the induction of the septic physiology around POH 4 and POH 8 in this model.

All animal deaths occurred by POH 56, with none after that, indicating that anti-HMGB1 antibodies did not merely delay death but conferred lasting protection against lethal sepsis.[12] Some control animals survived even without antibiotic treatment. We consider that those controls did well because of the adhesion of the intestines and intraabdominal fat tissue to the perforated cecum. Given that possible scenario, the rats treated with anti-HMGB1 antibodies might have provided time to allow the formation of such adhesions when serum HMGB1 concentrations were maintained below the lethal level, thereby resulting in a greater possibility of recovery from CLP-induced peritonitis.

HMGB1 has recently been recognized as an important late mediator in sepsis.[1–3,12] According to Wang *et al.*[3], in systemic inflammatory responses inflammatory cytokines such as TNFα and IL-1β appear in serum transiently, while HMGB1 appears later in the serum as a persistent response. This is the reason why HMGB1 is called a "late mediator". However, the timing of the initial elevation of serum HMGB1 concentrations differs according to the cause of the insults. In murine models of endotoxemia, serum HMGB1 was first detectable 8 h following the administration of an LD50 dose of endotoxin.[1,3] In a murine CLP model, serum HMGB1 levels began to increase significantly 18 h after the induction of peritonitis.[3,12] In murine liver ischemia-reperfusion models, serum HMGB1 concentrations began to elevate as early as 6 h after surgery.[22]

In the present study, serum HMGB1 concentrations of the controls, especially in animals that died, dramatically increased by POH 4, although other early-acting mediators, such as TNFα and IL-1β, could not be measured because of the limited volume of the blood samples. This result might partially be due to the difference in the cause of the insults and partially to the absence of antibiotic therapy. It is possible that inflammation in CLP rats not treated with antibiotics developed more rapidly than in those treated with antibiotics and, as a result, serum HMGB1 concentrations began to increase at time points earlier than 8 h after CLP surgery. Because macrophages stimulated by endotoxin *in vitro* began to produce HMGB1 4 h after the stimulation, this result did not conflict with the results reported in other studies.[1] Further investigations are required to define the relative phase of the elevation of HMGB1 in comparison with that of other cytokines in the modified CLP model.

We developed the CLP model without necrosis of the cecum by preserving the blood flow of the ileocolic artery and vein. It has been reported that HMGB1 is actively secreted by monocytes and macrophages following stimulation with lipopolysaccharide, TNF-α, or IL-1β, whereas it is passively released from necrotic cells.[1–3,9–11,23–25] HMGB1 secreted actively by the inflammatory cells has also been reported to be structurally different from the HMGB1 released passively by necrotic cells, which is a potent adjuvant.[11] Future studies may be necessary to elucidate possible differences in function between HMGB1 released from monocyte/macrophages and HMGB1 released from necrotic cells. Conventional CLP models develop both bacterial peritonitis and cecal necrosis, but the report of Yang *et al.*[12] on the effect of anti-HMGB1 antibodies in mice subjected to conventional CLP did not distinguish between the source of HMGB1. Consequently, the present study therefore provides novel insights into the function of HMGB1 since these experiments were performed in the absence of any necrosis.

In conclusion, anti-HMGB1 therapy appears to be effective in the treatment of severe sepsis. These results provide impetus to future investigations examining the kinetics of change in serum HMGB1 levels in response to various kinds of insults and also to studies aimed at elucidating the difference between the inflammatory response due to bacterial peritonitis and the inflammatory response due to necrosis.

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